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(54) **Food composition.**

(57) Food compositions, enteral preparations and pharmaceutical preparations containing an effective amount of mammalian milk or colostrum derived TGF-β2-like MGF for the modulation of MHC associated immune responses in the gastrointestinal tract of humans or animals.

EP 0 527 283 A1

Field of the Invention

This invention relates to the use of milk-derived polypeptides of the transforming growth factor beta family for the regulation of immune responses at the gut level associated with MHC (major histocompatibility complex).

This invention relates especially to the use of mammalian milk or colostrum derived TGF-β-like MGF (milk growth factor) for the preparation of a food composition, an enteral preparation or a pharmaceutical composition, as well as to a food composition or to an enteral preparation containing an effective amount of mammalian milk or colostrum derived TGF-β-like MGF.

Background of the Invention and Prior Art

Human and bovine milk contain many biologically active polypeptides including growth factors (West, D.W. Exp.Clin.Endocrinol. 8 145-146,1989). One of these factors, MGF (milk growth factor) was recently identified as identical to or having close homology to a member of the transforming growth factor beta (TGF-β) family, notably TGF-β2 (Cox D.A. et al. Eur. J. Biochem. 197 353-358, 1991). TGF-β is the general name for a family of polypeptides consisting of at least 5 distinct but closely related members, which have considerable structural and biological homologies (Roberts, A.B., et al. In: Peptide Growth Factors and their Receptors Vol. 1, pp. 419-472, Eds. Sporn M.B. et al., Springer, 1990). TGF-βs are homodimeric proteins of about 25 kDa consisting of identical 12.5 kDa polypeptide chains linked through disulphide bridges. They may form latent complexes with other proteins and these complexes may be activated by acid treatment or mild proteolysis (Roberts, A.B. et al.). They are multipotent, having a number of biological activities depending upon the target cell type, its state of differentiation and the presence of other factors. These activities include stimulation or inhibition of cell proliferation and differentiation, regulation of extracellular matrix deposition, immunomodulation, steroidogenesis and angiogenesis (Roberts, A.B. et al.).

Expression of MHC-Class II on the surface of antigen-presenting cells is a prerequisite for the presentation of exogenous antigen to T-cells (Benacerraf, B., Science 212 1229, 1981). Epithelial cells in the intestinal villus of the adult rodent constitutively express MHC-Class II while its expression by crypt cells depends in part on their spatial location in the intestine (Hughes, A., et al. Immunol. 72 491, 1991). In the postpartum period in the rodent there is little or no expression of MHC-Class II by enterocytes until after weaning, thus indicating the presence of a suppressive factor in milk (Hughes, A. et al.).

TGF-βs, including TGF-β2, have a number of immunoregulatory properties and act at several stages of the inflammatory and immune reaction. For example they inhibit the proliferation of T and B lymphocytes (Kerhl, J.H., et al. J.Immunol. 137:3855-3860, 1986; Kerhl, J.H., et al. J.Exp.Med. 163:1037-1050, 1986) and thymocytes (Ristow, H.J. Proc.Natl.Acad.Sci.USA 83 5531-5534,1986). They also antagonize the effects of interleukins including IL-1, IL-2 and IL-3 and other immunoregulatory agents such as tumor necrosis factor and interferons (Roberts, A.B. et al.). Although most of their effects on immune cells are inhibitory, TGF-βs appear to play a critical role in isotype switching of IgG and IgM secreting cells to IgA secreting cells (Lebman, D.A., et al. J.Immunol. 144:952-959, 1990). With particular reference to reported immunosuppressive effects of MGF, this factor has been shown to decrease the proliferation of human lymphocytes induced by anti-CD3 or interleukins (Stoeck, M., et al. FEBS Lett. 249 289-292,1989); Stoeck, M., et al. J.Immunol. 143 3258-3265, 1989). TGF-βs interfere with certain accessory cell functions important in antigen presentation and specifically were shown to suppress MHC-Class II expression by melanomas, glial cells and astrocytes (Czarniecki, C.W., et al J.Immunol. 140 4217-4223, 1988; Schlusener H.J. J.Neuroimmunol. 24 41-47, 1990; Zuber, P. et al. Eur.J.Immunol. 18 1623-1626,1988). However, the regulation of MHC-Class II expression on epithelial cells in the intestine by TGF-βs or MGF has not hitherto been reported.

Altered regulation of MHC-Class II has been implicated in several gastrointestinal disorders. The presence of active inflammation at the gut level generally results in an increase in MHC-Class II expression on human intestinal epithelium and lamina propria (Mayer, L., et al. Gastroenterology 100 3-12, 1991). This increase is a conspicuous component of Inflammatory Bowel Disease (IBD), (Mayer, L. et al.). In IBD, tissue damage is due either to an autoimmune attack on the cellular components of the host intestinal mucosa (Snook, J.A., et al. Gut 32 163-166, 1991), or to a disorder in the mucosal immune regulation with an overreactivity to luminal antigens in the gut, based on a defective down-regulation of this response (Challenges in IBD Research: Agenda for the 1990's. National Foundation for Ileitis and Colitis. Feb. 21, 1990. Washington D.C.).

Both possibilities imply the existence of a disregulation of the mucosal immune response and emphasize an immunologic role in the initiation and perpetuation of the inflammatory response.

Object of the Invention

The object of the present invention is to provide a food composition, an enteral preparation or a pharmaceutical preparation for regulating MHC mediated immune responses in the mammalian gastrointestinal tract, and more especially for the treatment of Inflammatory Bowel Diseases (e.g. Crohn's disease, Ulcerative Colitis)or Graft-vs-Host reactions in humans or animals, for the prevention of diarrhea in weaning humans or animals, or for the prevention of allergic reactions in the gastrointestinal tract in humans or animals.

Summary of the Invention

The food composition, the enteral preparation, and/or the pharmaceutical preparation according to the present invention contain an effective amount of mammalian milk or colostrum derived TGF-β2-like MGF for the modulation of MHC expression in the gastrointestinal tract of humans or animals; said amount being preferably effective for the treatment of Inflammatory Bowel Diseases (e.g. Crohn's disease, Ulcerative Colitis) or Graft-vs-Host reactions in humans or animals, for the prevention of diarrhea in weaning humans or animals, or for the prevention of allergic reactions in the gastrointestinal tract in humans or animals.

Detailed Disclosure of the Invention

For preparing the food composition or the enteral preparation, or for carrying out the uses according to the present invention, a bioactive milk component, identical to or with close homology to TGF-β2 may be prepared in an enriched form from mammalian milk products, especially from bovine milk products, e.g. as disclosed in EP-A1-313515 (CIBA-GEIGY AG) p. 6 l. 11 to p. 7 l. 34 and Examples 1 to 3, and having TGF-β2-like activity on the proliferation of mammalian liver epithelial cells and on the expression of MHC by mammalian intestinal epithelial cells. Henceforth this bioactive milk factor is termed TGF-β2-like MGF.

Test 1,TGF-βs in Milks

Normal rat liver epithelial (RLE) cells which have previously been shown to be sensitive to the growth inhibitory effects of TGF-βs (Huggett, A.C., et al. Cancer Res. 50 7468-7475, 1990) were incorporated into a bioassay for the analysis of TGF-βs in milks and in acid-treated milk fractions and milk powders. Measurement of inhibition of DNA synthesis by ^{3}H-Thymidine incorporation was performed as described previously (HuggettA.C. et al.). Antibodies raised against TGF-βs (British Bio-technology Ltd.) were coincubated with standards or samples prior to bioassay analysis in order to determine inhibitory activity specific to TGF-β isoforms. Using this assay a 50% inhibition of RLE cell DNA synthesis is obtained with 50 pg/ml of TGF-β1 or TGF-β2.

Human and bovine milk were delipidated by centrifugation, desalted on PD-10 columns (Pharmacia) eluted with PBS and then sterilized by filtration through 0.2μm membranes (Millipore). Protein contents were monitored using the method of Smith et al (Smith P.K., et al. Anal. Biochem. 150: 76-85, 1985). For analysis of latent acid-activatable TGF-βs, the milk samples were adjusted to pH 4 with 1N HCl, centrifuged at 40000 g for 60 min to separate whey and casein fractions which were then neutralized with 1N NaOH and dialyzed against PBS. Dilutions were then analyzed using the RLE cell bioassay together with a series of TGF-β standard solutions. An estimation of the amount of TGF-β-like activity was determined by a comparison of the degree of inhibition of DNA synthesis obtained with the samples against TGF-β standard curves. The identification of specific isoforms of TGF-β was determined by examining the effects of isoform-specific neutralizing antibodies on the inhibitory activity.

This test demonstrates that both human and cows milk contain acid-activatable TGF-β2-like MGF which is mainly associated with the casein fraction (Table 1).

Table 1

TGF-β2-like MGF activity in Milks	
Sample	Active TGF-β2-like MGF (µg/g protein)
Bovine Milk	< 0.01
Bovine Acid Casein	0.52
Human Milk*	< 0.2
Human Acid Casein	0.75

*This value is overestimated due to the large amounts of EGF in these samples which interfere with the assay.

Tests 2 and 3

Suppression of MHC-Class II Expression by Intestinal Epithelial Cells

The HT-29 intestinal epithelial line derived from human colonic epithelial cells (Fogh, J. et al. In: Human Tumor Cells "in vitro". J.Fogh, ed. Plenum Publishing Corp., New York, pp. 115, 1975), were maintained in an undifferentiated state in glucose-containing media (Zweibaum, A., et al. J.Cell.Physiol., 122: 21, 1985). When the cells reached 70-80% confluence, they were exposed, over a 48h period, to one of the following treatments:- human recombinant interferon-gamma (IFN-γ, 100 U/ml) alone (Boehringer Mannheim); IFN-γ in combination with TGF-β2; IFN-γ followed by TGF-β2; TGF-β2 alone followed by IFN-γ; or, as a control, culture media alone. Cells were washed and retreated after the first 24h. TGF-β2 was used at doses ranging from 0.05ng to 4ng per ml. Following the treatment period, the cells were washed, fixed and the plates stored frozen at -20 ° C until required.

The avidin-biotin complex method of immunoperoxidase staining (Cerf-Bensussan, N., et al. J.Immunol., 130: 2615, 1983) was performed on monolayers utilising the mouse monoclonal antibody L234 (Becton Dickinson), which recognises the human MHC-Class II histocompatibility antigen HLA-DR. Mouse myeloma IgG protein (Zymed) served as a control. In another series of experiments, a normal rat small intestinal cell line, IEC-18 (Quaroni, A., et al. J.Cell Biology, 80 248, 1979) was grown to 50% confluency and subjected to IFN-γ and/or TGF-β2 in the combinations listed above. Cells were then detached from the culture dishes using Versene (Life Technologies Ltd.) and stained, in suspension, using a standard, direct immunofluorescence technique. Briefly, cells were washed, incubated with normal serum for 5min and then with the FITC-conjugated mouse monoclonal antibody MRC OX-6 (Serotec) which recognises the rat Class II MHC antigen. Cells were then washed and fixed for at least 1h with 1% paraformaldehyde before analysis in the FACScan (Becton Dickinson).

During food allergy and inflammatory diseases, intestinal epithelial cells express high levels of Class II antigen thought to be mediated, at least in part, by inflammatory cytokines such as IFN-γ. The HT-29 undifferentiated cells employed in the assay described, do not constitutively express Class II molecules. To partially mimic events taking place during the onset of intestinal inflammation, the cells were exposed to IFN-γ. The effect of TGF-β2 on this reaction was then examined. Exposure to IFN-γ induced Class II expression on the HT-29 cells but this effect was abrogated by pretreatment with TGF-β2 at all the doses tested (Table 2). In contrast, the other combinations of cytokines tested resulted in high levels of Class II expression. The majority of IEC-18 cells already expressed Class II molecules but showed increased expression following treatment with IFN-γ (Table 3). Once again, TGF-β2 suppressed this induction. Thus, at the onset of inflammatory intestinal reactions, TGF-β2 may modulate local expression of Class II antigens.

Table 2

Effect of TGF-β2 on MHC-Class II expression by human intestinal epithelial cells (HT-29).		
Treatment		MHC-II Expression
(0-24h)	(24-48h)	
none	none	-
none	IFN-γ	+ +
IFN-	none	+ +
IFN-γ	IFN-γ	+ + +
TGF-β2	none	-
TGF-β2	TGF-β2	-
TGF-β2	IFN-γ	-
IFN-γ	TGF-β2	+ +
TGF-β2 + IFN-γ	TGF-β2 + IFN-γ	+ +
Staining: - negative + weak + + strong + + + very strong		

Table 3

Effect of TGF-β2 on MHC-Class II expression by rat intestinal epithelial cells (IEC-18).		
Treatment		MHC-II Expression (% positive cells)
(0-24h)	(24-48h)	
none	none	73.6 ± 1.5
none	IFN-γ	85.3 ± 5.3
IFN-γ	IFN-γ	95.8 ± 0.6
TGF-β2	none	67.3 ± 1.8
TGF-β2	IFN-γ	75.8 ± 0.3
TGF-β2 + IFN-γ	TGF-β2 + IFN-γ	86.9 ± 1.5

The demonstration of MCH-Class II antigens on human and rodent intestinal cells supports the notion that these cells may act as antigen presenting cells (Mayer, L., et al. J.Exp. Med. 166 1471-1483, 1987. The epithelial cell of the intestine has been considered a major participant in the etiopathogenesis of IBD. An increase in their expression of MHC-Class II could lead to an increased epithelial-T-helper lymphocyte interaction and this, in turn, could be a primary event in IBD or a perpetuating mechanism. The present studies demonstrate for the first time the action of TGF-β2 (and TGF-β2-like MGF) on suppression of MHC-Class II expression on intestinal epithelial cells. According to these findings, the availability of an immunosuppressive agent acting topically at the surface of the intestinal mucosa could provide a new tool to interrupt the pathogenic mechanism involved in IBD and other inflammatory-immune conditions in the gut, namely Coeliac Disease and Graft-vs-Host reactions.

Example 1

TGF-β2-like MGF prepared in enriched form from bovine milk as disclosed above is added to a nutritionally balanced enteral product comprising about 10% of dry matter in such a quantity that the enteral preparation thus obtained comprises an amount of about 0.1 to 50, preferably 0.5 to 20 µg of TGF-β2-like MGF per g of dry matter.

The enteral preparations prepared in this way are effective in suppressing MHC-Class II expression by intestinal epithelial cells.

Example 2

TGF-β2-like MGF prepared in enriched form from bovine milk as disclosed above is added to a balanced food product in liquid or powder form in such a quantity that the food composition thus obtained comprises an amount of about 0.1 to 50, preferably 0.5 to 20 µg of TGF-β2-like MGF per g of dry matter.

The food composition prepared in this way are effective in suppressing MHC-Class II expression by intestinal epithelial cells.

Claims

1. A food composition containing an effective amount of mammalian milk or colostrum derived TGF-β2-like MGF for the modulation of MHC expression in humans or animals.

2. Food composition according to claim 1, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the treatment of Inflammatory Bowel Diseases or Graft-vs-Host reactions in humans or animals.

3. Food composition according to claim 1, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the prevention of diarrhea in weaning humans or animals.

4. Food composition according to claim 1, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the prevention of allergic reactions in the gastrointestinal tract in humans or animals.

5. Use of mammalian milk or colostrum derived TGF-β2-like MGF for the preparation of a food composition according to any of claims 1 to 4.

6. An enteral preparation containing an effective amount of mammalian milk or colostrum derived TGF-β2-like MGF for the modulation of MHC expression in humans or animals.

7. Enteral preparation according to claim 6, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the treatment of Inflammatory Bowel Diseases or Graft-vs-Host reactions in humans or animals.

8. Enteral preparation according to claim 6, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the prevention of diarrhea in weaning humans or animals.

9. Enteral preparation according to claim 6, wherein said amount of mammalian milk or colostrum derived TGF-β2-like MGF is effective for the prevention of allergic reactions in the gastrointestinal tract in humans or animals.

10. Use of mammalian milk or colostrum derived TGF-β2-like MGF for the preparation of an enteral preparation according to any of claims 6 to 9.

11. Use of mammalian milk or colostrum derived TGF-β2-like MGF for the preparation of a pharmaceutical preparation containing an effective amount of mammalian milk or colostrum derived TGF-β2-like MGF for the modulation of MHC expression in humans or animals.

12. Use according to claim 11, wherein said pharmaceutical composition is for the treatment of Inflammatory Bowel Diseases or Graft-vs-Host reactions in humans or animals.

13. Use according to claim 11, wherein said pharmaceutical composition is for the prevention of diarrhea in weaning humans or animals.

14. Use according to claim 11, wherein said pharmaceutical composition is for the prevention of allergic reactions in the gastrointestinal tract in humans or animals.

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EUROPEAN SEARCH REPORT

Application Number

EP 91 81 0629

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	EP-A-0 313 515 (CIBA-GEIGY AG) * Whole document * ---	1-14	A23L1/305 A61K35/20 A61K37/02
D,Y	EUROPEAN JOURNAL OF BIOCHEMISTRY vol. 197, no. 2, April 1991, BERLIN, G pages 353 - 358; D.A.COX ET AL.: 'Isolation and characterization of milk growth factor, a transforming-growth-factor-beta2-related polypeptide, from bovine milk' * Whole article * ---	1-14	
Y	EP-A-0 269 408 (GENENTECH, INC.) * Whole document * ---	1-14	
A	WO-A-9 000 900 (AMGEN INC) * Whole document * ---	2,7,12	
A	US-A-4 440 860 (MICHAEL KLAGSBRUN) * Whole document , in particular column 1 lines 48 -65 * ---	1,6,11	
D,Y	IMMUNOLOGY vol. 72, no. 2, February 1991, OXFORD, GB pages 491 - 496; A. HUGHES ET AL: 'Expression of MHC class II (Ia) antigen by the neonatal enterocyte: the effect of treatment with interferon-gamma' * Whole article, in particular page 495 column 1 * ---	1-14	TECHNICAL FIELDS SEARCHED (Int. Cl.5) A23L A61K C07K
Y	CHEMICAL ABSTRACTS, vol. 107, no. 9, 31 August 1987, Columbus, Ohio, US; abstract no. 76199V, F. H. MORRISS JR.: 'Growth factors in milk' page 570 ;column 2 ; & Hum. Milk Infant Nutr. Health 1986, 98-114 * abstract * --- -/--	1-14	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	16 APRIL 1992	JULIA P.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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EUROPEAN SEARCH REPORT

Application Number

EP 91 81 0629

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	PEDIATRIC RESEARCH vol. 25, no. 4, April 1989, BALTIMORE, US page 269A; M. EL-YOUSSEF ET AL.: 'Identification of tumor necrosis factor alpha (TNF-alpha) and transforming growth factor beta (TGF-beta) in murine milk' * Abstract *	1,6,11	
A	WO-A-9 003 812 (GENENTECH, INC.) * Whole document *	2,7,12	
T	JOURNAL OF PROTEIN CHEMISTRY vol. 10, no. 5, October 1991, NEW YORK, US pages 565 - 575; Y. JIN ET AL.: 'Separation, purification, and sequence identification of TGF-beta1 and TGF-beta2 from bovine milk' * Whole article *	1,6,11	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

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BERLIN	16 APRIL 1992	JULIA P.

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